Targeted screen for functional interactions between β-tubulin and +TIP mutants in *Saccharomyces cerevisiae*

Honors Thesis
Presented to the College of Agriculture and Life Sciences,
Cornell University
in Partial Fulfillment of the Requirements for the
Biological Sciences Honors Program

by
Demitri Dedousis

Spring 2014

Research Supervisor: Professor Tim Huffaker
Thesis Advisor: Professor William J. Brown

Abstract

The microtubule cytoskeleton is involved in key cellular processes, including cellular polarization and transport. Microtubules are composed of repeating α and β tubulin dimers. *TUB2* is an essential yeast gene that encodes β-tubulin. Microtubule polymerization dynamics in yeast are regulated by +TIPs, like Bim1p and Bik1p. Genetic analysis of multiple genes concurrently allows for the identification of interactions between genes. This study seeks to investigate interactions between Bim1p/Bik1p and Tub2p by using random spore analysis (RSA) to search for synthetic interactions caused by the association of *bim1Δ/bik1Δ* and *tub2* mutants. Using the RSA results and a set of criteria, *tub2* mutants were grouped into interaction types, such as synthetic sensitive and synthetic enhanced. This study also investigated associations between a *tub2* mutation's interaction type and other *tub2* phenotypes, which revealed an association between synthetic sensitivity and cold sensitivity for *tub2 and bik1Δ*. A protein model of *tub2* mutations in β-tubulin was also used to demonstrate that *tub2* mutations that did not interact with *bim1Δ/bik1Δ* tended to cluster together in the same location on β-tubulin. This study shows that RSA can be used to identify synthetic interactions, which can be analyzed in terms of protein structure and relationship with other traits of the mutations.

Background Information

The microtubule cytoskeleton is involved in many key cellular processes. In addition to providing structure to the cell, microtubules also form the spindle that segregates chromosomes during mitosis, and are involved in cell polarization, migration and long range intracellular transport (Galjart, 2010). Microtubules are composed of α- and β-tubulin dimers that are organized in a head to tail fashion into protofilaments that, in turn, form the microtubule's hollow tube structure. Microtubules have a plus end and minus end; the minus end is usually embedded into a microtubule organizing center, while the plus end radiates outwards and explores the intracellular space by alternatingly growing and shrinking, a property known as dynamic equilibrium (Kirschner & Mitchison, 1986).

Dynamic equilibrium is caused by differences in the distribution of GTP between growing and shrinking microtubules. Tubulin molecules reversibly bind and hydrolyze one GTP molecule at their β subunit and irreversibly bind another GTP at their α subunit. The β-tubulin GTP is hydrolyzed concurrently with microtubule assembly. However, the rate of assembly exceeds the rate of hydrolysis, creating microtubules with unhydrolyzed GTP-tubulin caps at each end and hydrolyzed GDP-tubulin in their interiors. Microtubules with GTP caps grow, while microtubules without GTP caps shrink rapidly. (Kirschner & Mitchison, 1986)

Saccharomyces cerevisiae is a convenient model organism for studying microtubule function because it has relatively simple microtubule arrays (Jacobs, Adams, Szaniszlo, & John, 1988). Furthermore, there is only one yeast gene, TUB2, that encodes beta-tubulin, a protein 75% similar to animal beta-tubulin (Neff, Thomas, Grisafi, &

Botstein, 1983). Since TUB2 is an essential gene, an assortment of non-lethal "charged to alanine" alleles of this gene were used for this genetic analysis (Renee A. Reijo, Eric M. Cooper, Gwyneth J. Beagle, 1994). Charged to alanine mutations result from targeted mutagenesis that converts charged amino acids (glutamate, aspartate, lysine and arginine) to alanine, or in one case phenylalanine. Charged amino acids tend to be on the exterior of a protein; hence, this method focuses on residues likely to be involved in protein-protein interactions, while limiting the chance the mutations will affect protein folding.

Microtubules are regulated by a diverse group of proteins known as microtubule associated proteins (MAPS) (Amos & Schlieper, 2005; Galjart, 2010). The polymerization dynamics of microtubules are regulated by a subgroup of the MAPS called plus end tracking protein (+TIPs), which accumulate at the positive, or growing end, of the microtubule. +TIPs also link microtubules to other cellular structures, such as vesicles, the cell membrane, kinetochores, and other cytoskeletal structures (Galjart, 2010). +TIPs associate with and regulate one another, creating a complex web of interactions (Akhmanova & Hoogenraad, 2005).

A number of +TIPs are evolutionarily well conserved from yeast to humans, highlighting their importance. The human +TIP proteins EB1 and CLIP-170 are homologs of the yeast proteins Bim1p and Bik1p respectively (Amos & Schlieper, 2005; Schuyler & Pellman, 2001). EB1 and CLIP-170 are present on microtubules and take part in spindle orientation and chromosome segregation. EB1 and CLIP-170 also form stable homodimeric complexes together (Blake-Hodek, 2010).

In vitro Bim1p binds with tubulin to associate with the microtubule lattice, while Bik1p cannot bind tubulin and requires Bim1p to associate with the microtubule plus end.

In vitro Bim1p promotes microtubule growth while Bik1p inhibits growth; however, Bim1p and Bik1p together have a similar effect as Bim1p alone. This suggests that the association of Bim1p and Bik1p inhibits the effects of Bik1p while leaving Bim1p's effects unaltered. EB1 and Bim1p have similar in vitro effects on microtubules; both decrease shrinkage rates and promote microtubule growth. CLIP-170 has different in vitro effects than Bik1p; unlike Bik1p, it promotes microtubule polymerization (Blake-Hodek, 2010).

In vivo, Bim1p and Bik1p both track the plus ends of growing and shrinking microtubules and have redundant effects on microtubule dynamics. Microtubules are much less dynamic in cells without Bim1p and Bik1p. Like EB1 and CLIP-170, Bim1p and Bik1p interact in vivo to form a Bim1p-Bik1p tetramer complex that also regulates microtubule dynamics. Furthermore, *BIM1* and *BIK1* are both nonessential genes with functional overlap; yeast cells with either gene knocked out have similar phenotypes while double knockouts are lethal (Schwartz, Richards, & Botstein, 1997; Wolyniak et al., 2006).

Concurrent genetic analysis of multiple genes offers insight into genetic function and allows for the identification of interactions between genes. Synthetic genetic interaction occurs when a secondary genetic mutation enhances or represses the primary mutant phenotype. A synthetic lethal interaction occurs when the combination of two normally viable mutations results in a lethal phenotype. It also suggests that the two genes involved interact, are involved in similar pathways, and compensate for one another when one gene or the other is mutated (Hartman, Iv, Garvik, & Hartwell, 2001;

Hin, Tong, & Boone, 2005). This method of screening double mutants for synthetic interaction has been previously applied to *Saccharomyces cerevisiae* (Hin et al., 2005).

Given the important role of Bim1p and Bik1p in regulating microtubules, and that they have been previously shown to interact with Tub2p, (Blake-Hodek, 2010) this study will investigate interactions between *BIM1*/*BIK1* and *TUB2*. It seeks to identify synthetic interactions between *bim1Δ*/*bik1Δ* and *tub2* mutants and to categorize *tub2* mutants based on the type of synthetic interaction that occurred. The study also looks for possible associations of synthetic interaction type with various *tub2* mutant phenotype, as well as patterns in location of *tub2* mutations within tubulin based on their synthetic interaction type.

Materials and Methods

Media and Plates

YEPD plates contained 2% bacto agar, 1% yeast extract and 2% glucose.

MATa spore selection plates (SC minus histidine/arginine/lysine plus canavanine/thialysine/G418/uracil/leucine) contained 0.17% yeast nitrogen base without amino acids or ammonium sulfate, 0.1% monosodium glutamate, 0.2% amino-acids supplement powder mixture minus histidine/arginine/lysine, 2% bacto agar, 2% glucose, 50 mg/L canavanine, 50 mg/L thialysine, 200 μg/ml G418.

Enriched sporulation media contained 1% potassium acetate, 0.1% yeast extract, 0.05% glucose, 20 μg/ml uracil, 30 μg/ml leucine, 20 μg/ml histidine and 80 μg/ml methionine.

RSA diploid selection (SD minus uracil plus G418) plates contained 0.17% yeast nitrogen base without amino acids, 0.1% monosodium glutamate, 2% glucose, 2% bacto agar, 0.077% uracil dropout amino acid mix and 200 μg/ml G418.

RSA selection plates contained 0.17% yeast nitrogen base without amino acids or ammonium sulfate, 0.1% monosodium glutamate, 2% bacto agar, 2% of glucose. Media for plate 1 (SD minus histidine/arginine/lysine plus canavanine/thialysine) also contains 20 mg/ml adenine solution, 30 μg/ml leucine, 20 μg/ml uracil solution, 50 mg/L canavanine solution and 50 mg/L thialysine. Media for plate 2 (SD minus histidine /arginine/lysine plus canavanine/thialysine/G418) also contains 20 mg/ml adenine solution, 30 μg/ml leucine, 20 μg/ml uracil solution, 50 mg/L canavanine solution and 50 mg/L thialysine and 200 mg/L G418. Media for plate 3 (SD minus histidine /arginine/lysine/uracil plus canavanine/thialysine) also contains 20 mg/ml adenine solution, 30 μg/ml leucine, 50 mg/L canavanine solution and 50 mg/L thialysine. Media for plate 4 (SD minus histidine /arginine/lysine/uracil plus canavanine/thialysine/G418) also contains 20 mg/ml adenine solution, 30 μg/ml leucine, 50 mg/L canavanine solution and 50 mg/L thialysine and 200 mg/L G418.

Yeast Strains

The yeast strains used in this study are listed in Table 1. The two query strains CUY2157 and CUY2159 were created by crossing CUY2155 with CUY1817 and CUY1827 respectively. Diploids were obtained by streaking crosses onto SC minus methionine plus histidine/leucine/uracil to select against CUY1817 and CUY1827 haploids, and then onto YEPD plates containing 200 mg/L G418 to select against CUY2155 haploids. The product was sporulated in enriched sporulation media. The

spores were then plated on SC minus histidine arginine and lysine plus canavanine/thialysine/G418/uracil/leucine to select for the *MATa* spores. This yielded CUY2157 and CUY2159, which have *bim1*Δ and *bik1*Δ deletions, respectively, marked by KanMX.

Random Spore Analysis (RSA)

The two query strains CUY2157 and CUY2159 were mated with the *tub2* mutant strains. To select diploids for sporulation, the matings were streaked on SD minus uracil plus G418 plates. Diploids were grown to a density of ~1-2x10^8 cells/ml in YPED. 1 ml of YEPD was transferred to a clean tube and rinsed twice in sterile H_2O. The cells were pelleted and resuspended in 1 ml of enriched sporulation media. The sporulation tubes were then shaken at room temperature. Sporulation was confirmed through microscopy. Then 1 ml of each sporulation culture was transferred to fresh tubes and pelleted. 1 µl of the resulting cell slurry was resuspended in 1 ml of sterile H_2O in fresh tubes. Cells were then plated onto four types of selective plates, all of which selected against unsporulated diploid cells.

Cells were plated in different amounts to standardize the number of colonies expected on each type of plate (see Figure 9). 20 µl of spores were plated on plate 1 (SD minus histidine/arginine/lysine plus canavanine/thialysine); plate 1 and all subsequent plates selected for MATalpha spores to avoid further mating. 40 µl of suspended spores were plated on plate 2 (SD minus histidine/arginine/lysine plus canavanine/thialysine/G418). Plate 2 selected for spores with the KanMX gene; only spores with the *bik1*Δ(or *bim1*Δ)::KanMX construct grew on plate 2. 40 µl of suspended spores were plated on plate 3 (SD minus histidine/arginine/lysine/uracil plus

canavanine/thialysine). Plate 3 selected for spores with the uracil gene; only spores with the *tub2Δ::URA3* construct grew on plate 3. 80 µl of suspended spores were plated on plate 4 (SD minus histidine/arginine/lysine/uracil plus canavanine/thialysine/G418). Plate 4 selected for spores with both the *URA3* and KanMX genes; only spores with the *tub2Δ::URA3* and *bik1*Δ (or *bim1*Δ)::KanMX construct (double mutants) grew on plate 4. RSA plates were then grown at 30˚C for 2 to 3 days.

After RSA plates grew up, photographs of each plate were taken and the colonies were counted by hand with the assistance of a colony counter pen. RSA plates with greater then 500 colonies were assumed to have 500 colonies for calculation purposes, because at such a high colony density counting accurately becomes difficult. If more than one RSA trial for a *tub2* strain were available, the colony number and colony ratio for each plate was averaged.

Results

Experimental Design

Random Spore Analysis (RSA) was used to investigate the interactions between *tub2* mutants and *bim1*Δ*/bik1*Δ. First, the *tub2* mutant strains were mated with strains containing the *bim1*Δ or *bik1*Δ alleles. The diploid products of the matings were then sporulated. The spores were then plated on four different kinds of selective plates. The first plate and all subsequent plates selected for MATalpha spores to avoid further mating, the second plate selected for spores with *bim1*Δ*/bik1*Δ alleles, the third plate selected for spores with *tub2* mutants, and the fourth plate selected for spores with both *bim1*Δ*/bik1*Δ and *tub2* mutants. The number of colonies that grew on each type of plate

was then counted. The number of colonies on the fourth plate was then compared with the number of colonies on the other plates to categorize the *tub2* mutants by their interactions with *bim1*Δ*/bik1*Δ.

To standardize for the varying number of colonies in different trials, this study used the colony ratio, the number of colonies on a plate relative to the number of colonies on the first plate. In a *tub2* mutant strain that had no genetic interaction with *bim1*Δ*/bik1*Δ, the number of colonies on each plate would be similar and the colony ratio for each plate would be approximately 1. This is the case for strain CUY2169, which had a wildtype *TUB2* gene and served as a control (see first row of tables 3 and 5).

To isolate the interactive effects of *bim1*Δ*/bik1*Δ and *tub2* from the individual effects of *bim1*Δ*/bik1*Δ and *tub2* on the number of colonies on plate 4, this study used the expected colony ratio of plate 4. The expected colony ratio of plate 4 (exp. CR of P4) is the product of the colony ratio of plate 2 and plate 3. It is used to estimate what the colony ratio of plate 4 would have been if only the individual effects of *bim1*Δ*/bik1*Δ and the *tub2* mutation had been at play, with no interaction between them (see column 6 of tables 3 and 5).

RSA revealed synthetic interactions between *bim1*Δ and *tub2* mutants

RSA of *bim1*Δ and *tub2* mutants revealed that many strains did not have a similar number of colonies on each plate (see figure 6). Many strains had plates with colony ratios that were significantly less than 1. For instance, the *tub2-426* spores produced a similar number of colonies on plates 1, 2, and 3, but many fewer colonies on plate 4. Plate 4 had fewer colonies because an interaction between *tub2-426* and *bim1*Δ caused double mutant spores to have lower viability. Strain 423 had moderately fewer colonies

on plates 2 and 3 than on plate 1. Plate 2 had fewer colonies because the *bim1*Δ reduced spore viability. The same is true of plate 3, except the *tub2-423* mutation, rather than *bim1*Δ, reduced spore viability. Many of the strains with colony ratios less than 1 followed the patterns of either strain 426 or 423.

There were also strains with colony ratios significantly larger than 1. Strain 432 had many more colonies on plates 2 and 4 than on plate 1. The fact that plate 2 has more colonies than plate 1 suggests that *bim1*Δ somehow increased spore viability. Plate 4 had more colonies than plate 1 because an interaction between *tub2* and *bim1*Δ caused double mutant spores to have higher viability.

RSA revealed synthetic interactions between *bik1*Δ and *tub2* mutants

RSA of *bik1*Δ and *tub2* mutants showed similar results to the RSA of *bim1*Δ and *tub2* (see figure 7). Many strains had plates with colony ratios that were significantly less than 1. For instance, strain 434 had moderately fewer colonies on plates 2 and 3 than on plate 1, but had many fewer colonies on plate 4 compared to plate 1. Plate 2 had fewer colonies because the *bik1*Δ reduced spore viability. The same is true of plate 3, except that the *tub2-434* mutation reduced spore viability. Plate 4 had fewer colonies because an interaction between *tub2-434* and *bim1*Δ caused double mutant spores to have lower viability. Many of the strains with colony ratios less than 1 followed the patterns of strain 434. There were also strains with colony ratios much greater than 1, like strain 433, which had many more colonies on plate 4 than on plate 1. This is because an interaction between *tub2-433* and *bik1*Δ caused double mutant spores to have higher viability.

The *tub2* mutations were categorized based on their interaction with *bim1*Δ/*bik1*Δ

The *tub2* strains were then sorted into different interaction types based on their colony ratios. The interaction types are synthetic sensitive (SS), synthetic lethal (SL), synthetic enhanced (SE) and no interaction (see figure 9). The synthetic sensitive class is for strains where a genetic interaction between *bim1*Δ/*bik1*Δ and a *tub2* mutation resulted in fewer colonies on plate 4 than on plate 1. To be classified as synthetic sensitive, a *tub2* strain had to have a colony ratio of plate four that was less than 0.8, less than the colony ratio of plate 2 and plate 3, and also less than the expected colony ratio of plate 4.

The maximum colony ratio of plate 4 was set at 0.8 to ensure that the number of colonies on plate 4 was significantly different from the number of colonies on plate 1. The colony ratio of plate 4 also had to be less than the ratio of plates 2 and 3 to ensure that the individual effects of *bim1*Δ/*bik1*Δ or the *tub2* mutation were not responsible for the lower number of colonies on plate 4. Requiring plate 4's colony ratio to be less than its expected colony ratio of plate 4 ensured that genetic interactions between *bim1*Δ/*bim1*Δ and the *tub2* mutation were responsible for the reduced number of colonies on plate 4, and not merely the aggregated individual effects of the *bim1*Δ/*bim1*Δ and the *tub2* mutation. The synthetic sensitive class was further subdivided into synthetic sensitive where the colony ratio of plate 4 is greater than 0.15 (SS > 0.15) and synthetic sensitive where the colony ratio of plate 4 is less than 0.15 (SS < 0.15) .

The synthetic enhanced class is defined as strains where a genetic interaction between *bim1*Δ/*bim1*Δ and a *tub2* mutation resulted in a greater number of colonies on plate 4 than on plate 1. To be classified as synthetic enhanced, a *tub2* strain had to have a colony ratio of plate 4 greater than 1.2, greater than the colony ratio of plate 2 and plate 3, and also greater than the expected colony ratio of plate 4. These criteria were chosen

for the same reasons as the criteria for the synthetic sensitive class. For instance, the colony ratio of plate 4 had to be greater than 1.2 to ensure that the number of colonies on plate 4 was significantly different from the number of colonies on plate 1.

The no interaction category is for strains where the number of colonies on plate 4 was either similar to the number of colonies on plate 1 or differed as a result of the individual effects of *bim1*Δ/*bik1*Δ or *tub2*. All strains that did not meet the criteria for synthetic sensitive or synthetic enhanced were classified as no interaction.

Most *tub2* mutations had the same type of interaction with both *bim1*Δ and *bik1*Δ

The interaction of *tub2* mutations with both *bim1*Δ and *bik1*Δ usually had similar effects on the number of colonies on plate 4 (see figure 8). Of the 20 *tub2* mutations for which RSA data was available for both *bim1*Δ and *bik1*Δ (no data was available for interaction of *tub2-436* and *tub2-438* with *bim1*Δ), 75% had the same classification when interacting with both *bim1*Δ and *bik1*Δ (compare the interaction columns of tables 3 and 5).

Several *tub2* mutation strains had different interaction types with *bim1*Δ and *bik1*Δ. Strain C12A had a synthetic enhanced interaction with *bim1*Δ and no interaction with *bik1*Δ, but its interaction with *bim1*Δ barely qualified as synthetic enhanced, and its interaction with *bik1*Δ nearly qualified as synthetic enhanced. Several *tub2* mutations (419, 428, 432, 441) that had a synthetic sensitive interaction and one *tub2* mutation that had a synthetic enhanced interaction with *bim1*Δ or *bik1*Δ had no interaction with the other deletion. No *tub2* mutations had a synthetic sensitive interaction with *bim1*Δ or *bik1*Δ and a synthetic enhanced interaction with the other.

Interaction type may be correlated with cold sensitivity in *tub2* mutations

This study also investigated possible associations between the interaction types of *tub2* mutations with *bim1*Δ/*bik1*Δ and other phenotypes of the *tub2* mutations (see table 10). The *tub2* mutant phenotypes are: cold sensitivity (CS), benomyl resistance (Ben-R), benomyl super sensitivity (Ben-SS) and slow growth. It was assumed that if there were no correlation, the phenotypes would associate randomly with interaction type. Goodness of fit for a random distribution of phenotypes was tested using a X^2 test ($P < 0.05$).

The X^2 test showed that interaction type for *bim1*Δ and *tub2* mutants randomly associated with each of the *tub2* phenotypes. But for *bik1*Δ and *tub2*, cold sensitivity associated non-randomly with interaction type ($p = 0.043$) (see table 10). A higher than expected proportion of synthetic sensitive strains were also cold sensitive (82%), with 45% of synthetic sensitive strains expected to be cold sensitive assuming a random distribution. This suggests a correlation between cold sensitivity and synthetic sensitive interactions between *bik1*Δ and *tub2*.

Pymol model of β-tubulin shows clustering of non-interacting *tub2* mutations

The protein modeling program Pymol was used to construct a model of β-tubulin that shows the amino acids affected by each *tub2* mutation (see figure 11, panels A and B). The *tub2* mutations were then grouped by their interaction type with *bim1*Δ (see figure 11 panels C and D) and *bik1*Δ (see figure 11 panels E and F). These images reveal a clustering of *tub2* mutations that did not interact with *bim1*Δ (see figure 11, panel D). To a lesser extent, there is also a clustering of *tub2* mutations that did not interact with *bik1*Δ (see figure 11, panel F). It is also interesting to note that strains 432 and 433, which both have synthetic enhanced interactions with *bim1*Δ, have amino acid replacements that are adjacent to one another (see figure 11 panel D blowup).

Discussion

Synthetic interactions between *bim1*Δ and *tub2* mutants

RSA has revealed synthetic sensitive and synthetic enhanced interactions between *bim1*Δ and particular *tub2* mutants. Synthetic sensitive interactions suggest that yeast cells containing both *bim1*Δ and a particular *tub2* mutation had higher lethality rates then cells with either mutation alone. It has been shown that *bim1*Δ make microtubules much less dynamic (Wolyniak et al., 2006); therefore, many of the *tub2* mutations that have synthetic sensitive interactions with *bim1*Δ may also reduce microtubule dynamics. The synthetic enhanced interactions suggest that the *bim1*Δ and particular *tub2* mutations interact to cause a phenotype that increases the fitness of the double mutant.

The analysis of possible relationships between mutation interaction type and other factors affecting yeast growth showed no evidence of association between interaction type and any of the factors investigated. Analysis of the β-tubulin mutation model showed that *tub2* mutations that did not interact with *bim1*Δ (see figure 11, panel D) clustered together on the exterior of the β-tubulin protein. This suggests either that Bim1p does not bind to β-tubulin where this clustering occurs, or that this section of the β-tubulin interacts differently with Bim1p than the rest of the domain. There did not appear to be any particular pattern to the location of the synthetic sensitive or synthetic enhanced mutations. However, the fact that these *tub2* mutations interacted with *bim1*Δ suggests that Bim1p may bind or otherwise interact with these amino acids.

Synthetic interactions between *bik1*Δ and *tub2* mutants

RSA also revealed synthetic sensitive and synthetic enhanced interactions between *bik1*Δ and particular *tub2* mutants. Synthetic sensitive interactions suggest that

yeast cells containing both *bik1*Δ and a particular *tub2* mutation had higher lethality rates than cells with either mutation alone. As with *bim1*Δ, it has been shown that *bik1*Δ makes microtubules much less dynamic (Wolyniak et al., 2006). Many of the *tub2* mutations that have synthetic sensitive interactions with *bik1*Δ may also reduce microtubule dynamics. This could explain why 75% of *tub2* mutants had the same interaction classification when interacting with either *bim1*Δ or *bik1*Δ. Both *bim1*Δ and *bik1*Δ reduce microtubule dynamics; consequently, they would both have synthetic sensitive interactions with the same *tub2* mutants, which also reduce microtubule dynamics.

There were also a small number of *tub2* mutants that interacted differently with *bim1*Δ and *bik1*Δ. This could be because a *tub2* allele that eliminates the ability of Tub2p to interact with Bim1p would not be synthetic sensitive with *bim1*Δ because the interaction between *TUB2* and *BIM1* was already broken. Such alleles would likely be synthetic sensitive with bik1Δ because *bim1*Δ and *bik1*Δ are synthetic lethal with each other (Wolyniak et al., 2006) and these *tub2* alleles are in effect *bim1* minus. This could explain the handful of strains that were synthetic sensitive with either *bim1*Δ/*bik1*Δ but had no interaction with the other.

The analysis of possible relationships between mutation interaction type and other *tub2* phenotypes revealed an association between synthetic sensitivity and cold sensitivity. This suggests that some of the same factors that render a strain cold sensitive are the same factors that make it more likely to have a synthetic sensitive interaction with *bik1*Δ. Analysis of the β-tubulin Pymol mutation model showed that *tub2* mutations that did not interact with *bik1*Δ (see figure 11, panel F) clustered together on the exterior of the β-tubulin protein, though not as tightly as with *bim1*Δ. This suggests that Bik1p does

not bind to β-tubulin where the clustering occurs. As with *bim1*Δ, there did not appear to be any particular pattern to the location of the synthetic sensitive or synthetic enhanced mutations. With that said, the fact that these *tub2* mutations interacted with *bik1*Δ suggests that the Bik1p may bind or otherwise interact with these mutated amino acids.

RSA Technique Limitations

Though the findings generated by RSA technique are fairly robust, there are concerns that should be addressed. The first is assuming that RSA plates with greater than 500 colonies had 500 colonies for calculation purposes. This allowed for a conservative estimate of the number of colonies on a plate that was too dense to count accurately. However, it also makes plates for RSA of certain strains (for *bim1*Δ: 429, 440, 441 and for *bik1*Δ: 408, 421, 426, 438, 441) appear as if they have an equal number of colonies when they do not (see figures 6 and 7). Second, tetrads were not separated into individual spores before they were plated on RSA selection plates. Therefore some of the colonies counted on the random spore analysis plates could in reality represent the growth of two spores.

Third, for some *tub2* mutations strains, RSA with *bim1*Δ and or *bik1*Δ resulted in the colony ratio of plate 2 or plate 3 being much larger than plate 1's ratio (see figures 6 and 7). Initially, this suggested that the presence of a *bim1*Δ/*bik1*Δ deletion or *tub2* mutation would improve the spore viability and increase the number of colonies. But this did not occur uniformly across the strains analyzed. There are three possible explanations for this phenomenon. One explanation is that the presence of *bim1*Δ/*bik1*Δ deletion or *tub2* mutation does increase spore viability. The second is that inaccurate counting or errors executing the protocol skewed the number of colonies on plates 2 and/or 3. The

third is that for *tub2* mutation strains that showed synthetic enhanced interaction, the double mutants present on plates 2 and 3 had increased spore viability, which increased the colony ratio values. If the second explanation were true, the relevance of all the synthetic enhanced interactions would be thrown into question.

Despite these limitations, RSA facilitates the identification of strains that have an synthetic interaction, especially for synthetic sensitive interactions, because of the dramatic change in the number of double mutant colonies on plate 4 (see figure 9). This study shows that RSA can be used to identify synthetic interactions between mutations, and that the interactions can then be grouped and analyzed in terms of their protein structure and relationship with other traits of the mutations.

Acknowledgment

I would like to thank Professor Tim Huffaker for his assistance in planning and executing this study, as well as for his comments on the manuscript. I would also like to thank Beth LaLonde for her assistance especially with regards to technical difficulties. Finally, I would like to thank my peers, Christine McIntosh and Stephen Sammons, for their support and helpful research suggestions.

Citations

Akhmanova, A., & Hoogenraad, C. C. (2005). Microtubule plus-end-tracking proteins: mechanisms and functions. *Current Opinion in Cell Biology*, *17*(1), 47–54. doi:10.1016/j.ceb.2004.11.001

Amos, L. a, & Schlieper, D. (2005). Microtubules and maps. *Advances in Protein Chemistry*, *71*(04), 257–98. doi:10.1016/S0065-3233(04)71007-4

Blake-Hodek, K. (2010). Regulation of microtubule dynamics by Bim1 and Bik1, the budding yeast members of the EB1 and CLIP-170 families of plus-end tracking proteins. *Molecular Biology of the …*, *21*, 2013–2023. doi:10.1091/mbc.E10

Galjart, N. (2010). Plus-end-tracking proteins and their interactions at microtubule ends. *Current Biology : CB*, *20*(12), R528–37. doi:10.1016/j.cub.2010.05.022

Hartman, J. L., Iv, J. L. H., Garvik, B., & Hartwell, L. (2001). Principles for the Buffering of Genetic Variation, *1001*(2001). doi:10.1126/science.1056072

Hin, A., Tong, Y., & Boone, C. (2005). 16 High-Throughput Strain Construction and Systematic Synthetic Lethal Screening in Saccharomyces cerevisiae. doi:10.1016/S0580-9517(06)36016-3

Jacobs, C. W., Adams, A. E. M., Szaniszlo, P. J., & John, R. (1988). Saccharomyces cerevisiae, *107*(October), 1409–1426.

Kirschner, M., & Mitchison, T. (1986). Beyond self-assembly: from microtubules to morphogenesis. *Cell*, *45*(3), 329–42. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/3516413

Neff, N. F., Thomas, J. H., Grisafi, P., & Botstein, D. (1983). Isolation of the beta-tubulin gene from yeast and demonstration of its essential function in vivo. *Cell*, *33*(1), 211–9. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/6380751

Renee A. Reijo, Eric M. Cooper, Gwyneth J. Beagle, and T. C. H. (1994). Systematic mutational analysis of the yeast beta-tubulin gene. *Molecular Biology of the Cell*, *5*(2), 29–43. Retrieved from http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3079708&tool=pmcentrez&rendertype=abstract

Schuyler, S. C., & Pellman, D. (2001). Proteins ": The End Is Just the Beginning, *105*, 421–424.

Schwartz, K., Richards, K., & Botstein, D. (1997). BIM1 encodes a microtubule-binding protein in yeast. *Molecular Biology of the Cell*, *8*(12), 2677–91. Retrieved from

http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=25736&tool=pmcentrez&rendertype=abstract

Wolyniak, M. J., Blake-hodek, K., Kosco, K., Hwang, E., You, L., & Huffaker, T. C. (2006). The Regulation of Microtubule Dynamics in Saccharomyces cerevisiae by Three Interacting Plus-End Tracking Proteins □, *17*(June), 2789–2798. doi:10.1091/mbc.E05

Tables and Figures

Table 1: Yeast strains used in study

CUY Strain	*tub2* Allele	AA Replaced	Genotype
640	408	R2A, E3A	MATalpha tub2-408::URA3ade2-101his3-Δ200leu2-Δ1lys2-801ura3-52
654	412	K58A, R62A	MATalpha tub2-412::URA3ade2-101his3-Δ200leu2-Δ1lys2-801ura3-52
677	418	E108A, E111A	MATalpha tub2-418::URA3ade2-101his3-Δ200leu2-Δ1lys2-801ura3-52
680	419	D114A, D118A	MATalpha tub2-419::URA3ade2-101his3-Δ200leu2-Δ1lys2-801ura3-52
685	420	R121A, R122A	MATalpha tub2-420::URA3ade2-101his3-Δ200leu2-Δ1lys2-801ura3-52
688	421	E123A	MATalpha tub2-421::URA3ade2-101his3-Δ200leu2-Δ1lys2-801ura3-52
696	423	I152F, K154A, R156A	MATalpha tub2-423::URA3ade2-101his3-Δ200leu2-Δ1lys2-801ura3-52
705	426	R156K, E157A	MATalpha tub2-426::URA3ade2-101his3-Δ200leu2-Δ1lys2-801ura3-52
710	428	D161A, R162A	MATalpha tub2-428::URA3ade2-101his3-Δ200leu2-Δ1lys2-801ura3-52
715	429	K174A, D177A	MATalpha tub2-429::URA3ade2-101his3-Δ200leu2-Δ1lys2-801ura3-52
726	432	E194A, D197A	MATalpha tub2-432::URA3ade2-101his3-Δ200leu2-Δ1lys2-801ura3-52
729	433	E198A	MATalpha tub2-433::URA3ade2-101his3-Δ200leu2-Δ1lys2-801ura3-52
733	434	D203A, E205A	MATalpha tub2-434::URA3ade2-101his3-Δ200leu2-Δ1lys2-801ura3-52
741	436	R213A, K216A	MATalpha tub2-436::URA3ade2-101his3-Δ200leu2-Δ1lys2-801ura3-52
748	438	R282A, E288A	MATalpha tub2-438::URA3ade2-101his3-Δ200leu2-Δ1lys2-801ura3-52
754	440	D304A, R306A	MATalpha tub2-440::URA3ade2-101his3-Δ200leu2-Δ1lys2-801ura3-52
757	441	R309A	MATalpha tub2-441::URA3ade2-101his3-Δ200leu2-Δ1lys2-801ura3-52
761	442	R318A, K320A	MATalpha tub2-442::URA3ade2-101his3-Δ200leu2-Δ1lys2-801ura3-52
796	452	E401A, D404A	MATalpha tub2-452::URA3ade2-101his3-Δ200leu2-Δ1lys2-801ura3-52
1817			MATa his3Δ1 leu2Δ0ura3Δ0met15Δ0bim1Δ::KanMX
1827			MATa his3Δ1 leu2Δ0ura3Δ0met15Δ0bik1Δ::KanMX
2155			MATalpha can1Δ::STE2pr-Sp_HIS5lyp1Δ::STE3pr-LEU2his3Δ1leu2Δ0ura3Δ0
2157			MATa bim1Δ::KanMXlyp1Δ::STE3pr-LEU2can1Δ::STE2pr-HIS5pombehis3Δ1leu2Δ0ura3Δ0
2159			MATa bik1Δ::KanMXlyp1Δ::STE3pr-LEU2can1Δ::STE2pr-HIS5pombehis3Δ1leu2Δ0ura3Δ0
2169			MATalpha TUB2::URA3ade2-101his3-Δ200leu2-Δ1lys2-801ura3-52
2173	C12A	C12A	MATalpha tub2-C12A::URA3ade2-101his3-Δ200leu2-Δ1lys2-801ura3-52
2176	E27A	E27A	MATalpha tub2-E27A::URA3ade2-101his3-Δ200leu2-Δ1lys2-801ura3-52
2177	R241F	R241F	MATalpha tub2-R241F::URA3ade2-101his3-Δ200leu2-Δ1lys2-801ura3-52

Table 1: A complete list of strains used in the study.

Table 2: Average Number of Colonies on Plates for RSA of the interaction between *bim1Δ* and *tub2*

# Trials	Strain	Plate 1	Plate 2	Plate 3	Plate 4
1	2169	771	874	714	816
1	408	68	4	7	2
1	412	207	188	130	12
2	418	345	347	157	11
2	419	135	177	92	88
2	420	222	246	185	246
1	421	475	500	484	483
3	423	269	199	126	10
1	426	286	282	254	10
2	428	183	63	64	19
1	429	500	500	500	67
2	432	279	360	241	430
1	433	244	286	225	322
1	434	107	162	85	2
2	440	500	500	500	500
1	441	500	500	500	134
1	442	140	371	301	302
2	452	185	132	126	52
1	C12A	191	309	215	353
1	E27A	72	57	76	3
1	R241F	161	233	198	78

Table 2: Column Labels: Plate 1= Non-selective towards spores, Plate 2= Selects for spores with *bim1Δ* (or *bik1Δ*)::KanMX, Plate 3= Selects for spores with *tub2*::URA, Plate 4= Selects for spores with both *tub2*::URA and *bim1Δ* (or *bik1 Δ*)::KanMX (double mutants). If multiple trials were available the average number of colonies for each plate was calculated. For plates where the number of colonies exceeded 500, 500 was used for calculations.

Table 3: Average Colony Ratio of Plates for RSA of interaction between *bim1Δ* and *tub2*

Strain	CR Plate 1	CR Plate 2	CR Plate 3	CR Plate 4	Exp. CR P4	Interaction
2169	1	1.13	0.93	1.06	1.05	None
408	1	0.06	0.10	0.03	0.01	None
412	1	0.91	0.63	0.06	0.57	SS
418	1	1.00	0.45	0.03	0.45	SS
419	1	1.34	0.72	0.82	0.96	None
420	1	1.00	0.81	1.08	0.81	None
421	1	1.05	1.02	1.02	1.07	None
423	1	0.78	0.45	0.04	0.35	SS
426	1	0.99	0.89	0.03	0.88	SS
428	1	0.44	0.44	0.09	0.19	SS
429	1	0.84	0.84	0.11	0.71	SS
432	1	1.29	0.82	1.56	1.06	SE
433	1	1.17	0.92	1.32	1.08	SE
434	1	1.51	0.79	0.02	1.20	SS
440	1	1.00	1.00	1.00	1.00	None
441	1	1.00	1.00	0.27	1.00	SS
442	1	2.65	2.15	2.16	5.70	None
452	1	0.70	0.64	0.26	0.44	SS
C12A	1	1.62	1.13	1.85	1.82	SE
E27A	1	0.79	1.06	0.04	0.84	SS
R241F	1	1.45	1.23	0.48	1.78	SS

Table 3: Column Labels: CR= Colony Ratio=number of colonies on plate x/number of colonies on plate 1, Exp. CR P4= Expected Colony ratio for plate 4= CR of plate 3 x CR plate 4. Interaction column: SS=Synthetic Sensitive, and SE=Synthetic Enhanced. Where more then one trial was available the average CR was calculated.

Table 4: Average Colony Ratio of Plates for RSA of interaction between *bik1Δ* and *tub2*

# Trials	Strain	Plate 1	Plate 2	Plate 3	Plate 4
1	2169	643	749	676	679
1	408	500	500	500	500
1	412	127	119	33	12
3	418	264	248	111	12
2	419	77	97	27	4
2	420	277	300	184	279
2	421	459	500	500	500
1	423	303	290	199	10
1	426	500	500	399	364
1	428	28	9	8	10
1	429	362	489	217	71
1	432	152	203	396	356
1	433	316	322	297	419
1	434	33	14	15	0
1	436	45	43	21	26
1	438	500	500	306	33
1	440	271	500	379	451
1	441	500	500	500	500
1	442	182	98	177	153
2	452	215	193	153	64
2	C12A	280	171	217	161
1	E27A	290	264	265	24
1	R241F	91	129	66	4

Table 4: Column Labels: Plate 1= Non-selective towards spores, Plate 2= Selects for spores with *bik1Δ*::KanMX, Plate 3= Selects for spores with *tub2*::URA, Plate 4= Selects for spores with both *tub2*::URA and *bim1Δ* (or *bik1*Δ)::KanMX (double mutants). If multiple trials were available the average number of colonies for each plate was calculated. For plates where the number of colonies exceeded 500, 500 was used for calculations.

Table 5: Average Colony Ratio of Plates for RSA of interaction between *bik1Δ* and *tub2*

Strain	CR Plate 1	CR Plate 2	CR Plate 3	CR Plate 4	Exp. CR P4	Interaction
2169	1	1.16	1.05	1.06	1.22	None
408	1	1.00	1.00	1.00	1.00	None
412	1	0.94	0.26	0.09	0.24	SS
418	1	0.97	0.37	0.05	0.36	SS
419	1	1.23	0.42	0.06	0.52	SS
420	1	1.08	0.66	1.00	0.71	None
421	1	1.10	1.10	1.10	1.21	None
423	1	0.96	0.66	0.03	0.63	SS
426	1	1.00	0.80	0.73	0.80	SS
428	1	0.32	0.29	0.36	0.09	None
429	1	1.35	0.60	0.20	0.81	SS
432	1	1.34	2.61	2.34	3.48	None
433	1	1.02	0.94	1.33	0.96	SE
434	1	0.42	0.45	0.00	0.19	SL
436	1	0.96	0.47	0.58	0.45	None
438	1	1.00	0.61	0.07	0.61	SS
440	1	1.85	1.40	1.66	2.58	None
441	1	1.00	1.00	1.00	1.00	None
442	1	0.54	0.97	0.84	0.52	None
452	1	0.89	0.73	0.33	0.65	SS
C12A	1	1.20	1.16	1.26	1.39	None
E27A	1	0.91	0.91	0.08	0.83	SS
R241F	1	1.42	0.73	0.04	1.03	SS

Table 5: Column Labels: CR= Colony Ratio=number of colonies on plate x/number of colonies on plate 1, Exp. CR P4= Expected Colony ratio for plate 4= CR of plate 3 x CR plate 4. Interaction column: SS=Synthetic Sensitive, and SE=Synthetic Enhanced. Where more then one trial was available the average CR was calculated.

Figure 6: RSA of interactions between *bim1Δ* and *tub2*

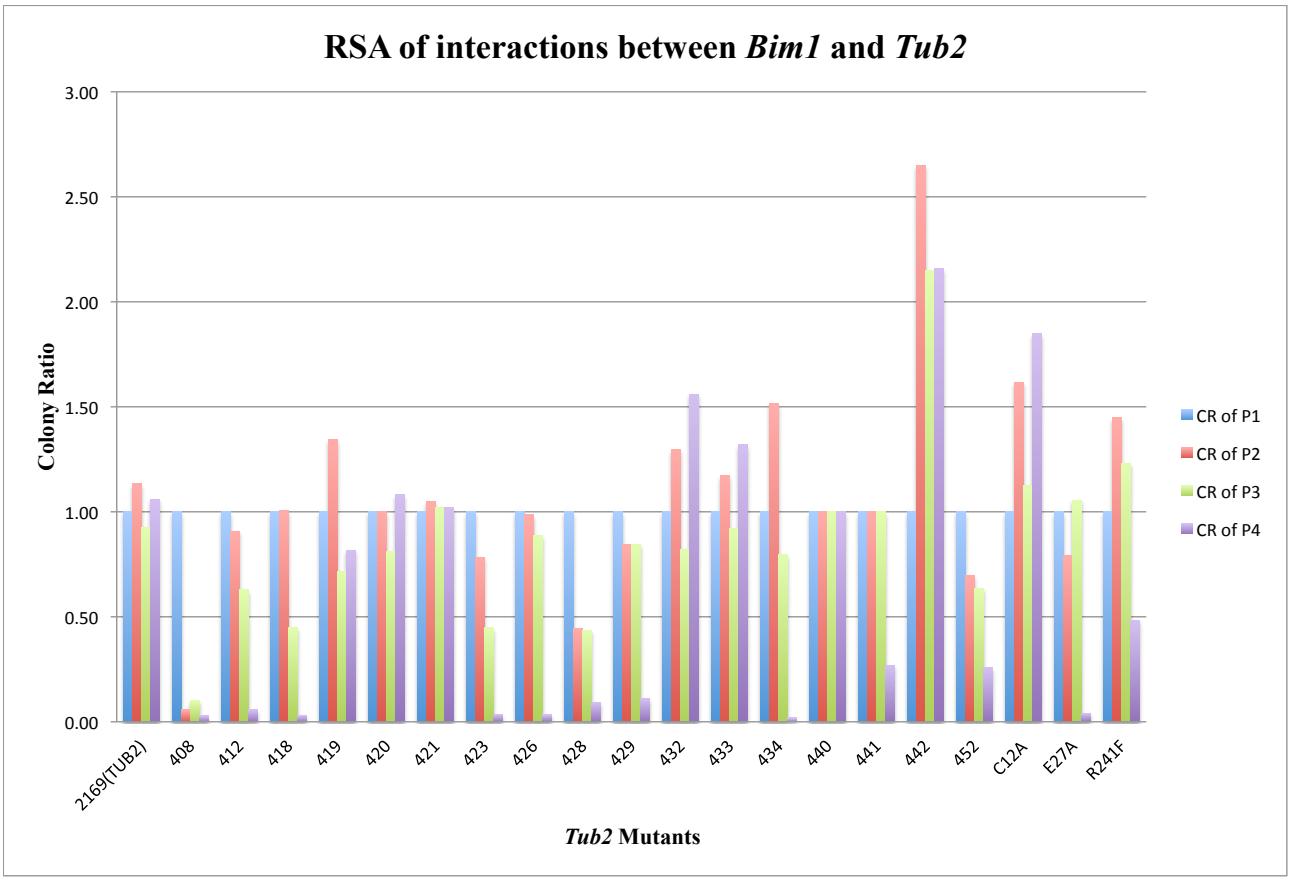


Figure 6: This set of RSA investigated the interactions between a *bim1Δ* and various *tub2* mutations. The graph shows the colony ratio (CR) of each plate (P1, 2, 3 and 4) for each *tub2* mutation.

Figure 7: RSA of interactions between *bik1Δ* and *tub2*

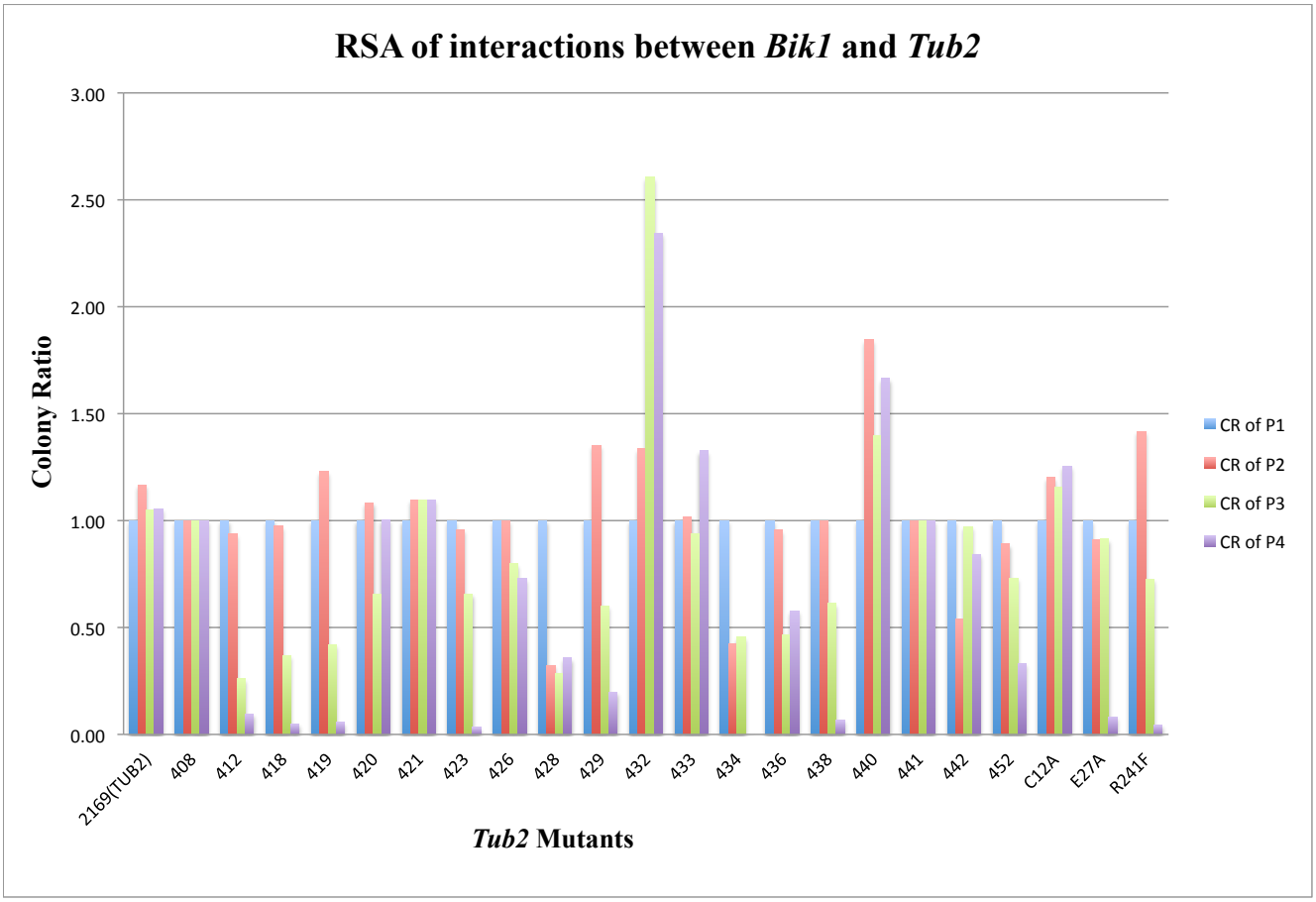


Figure 7: This set of RSA investigated the interactions between a *bim1***Δ** and various *tub2* mutations. The graph shows the colony ratio (CR) of each plate (P1, 2, 3 and 4) for each *tub2* mutation.

Figure 8: Comparison of *bim1Δ* and *bik1Δ* Synthetic Interactions with *tub2*

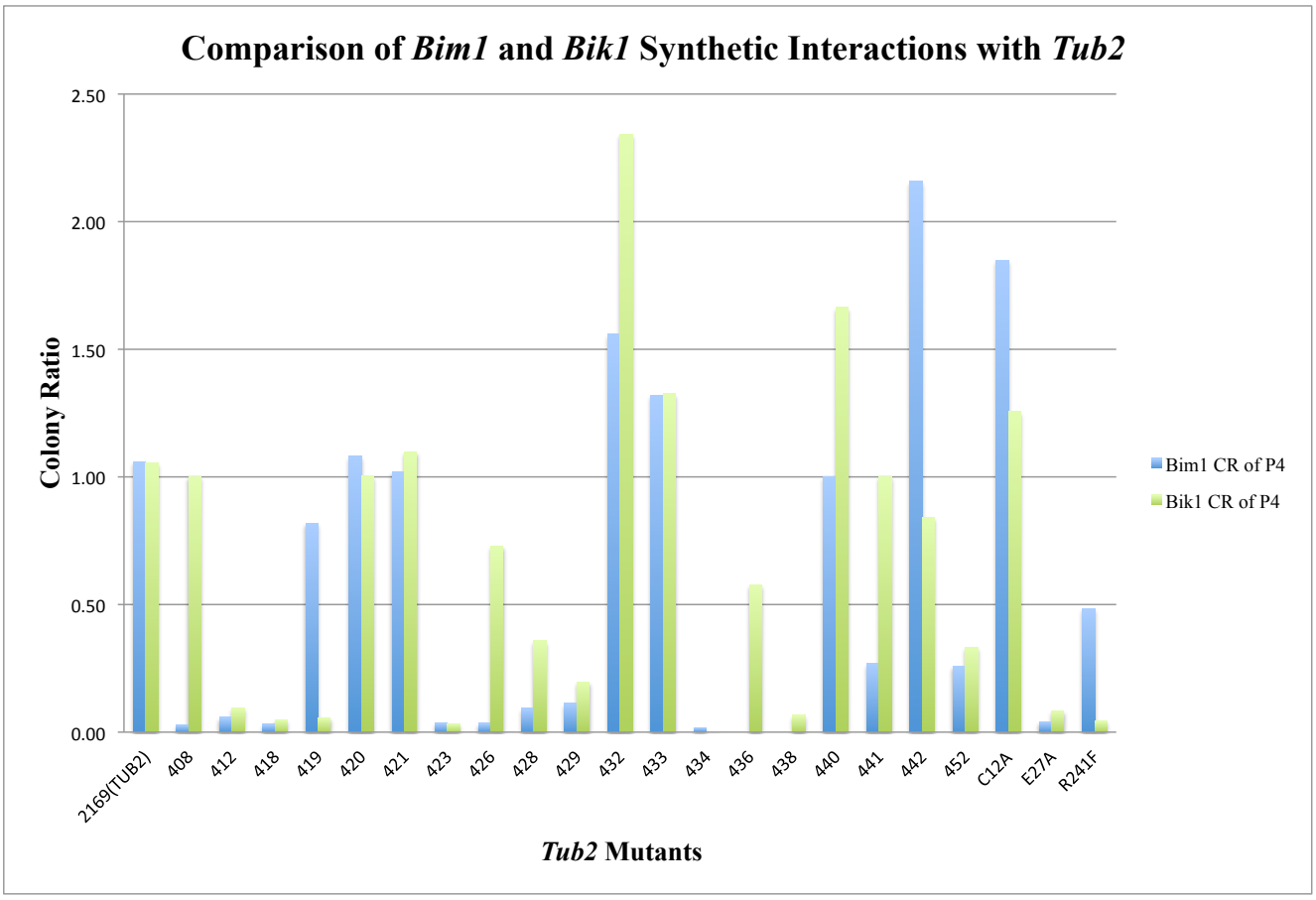


Figure 8: This graph compares the synthetic interactions of each *tub2* mutation with *bim1Δ* and *bik1Δ* by comparing the colony ratio (CR) values of Plate 4 (P4), the most selective plate, from *bim1Δ* and *bik1Δ* RSA. Also present for comparison are the expected values of the colony ratio for Plate 4.

Figure 9: Examples of RSA Plates Synthetic Sensitive and Synthetic Enhanced Interactions

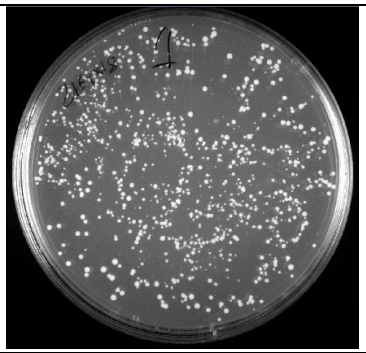
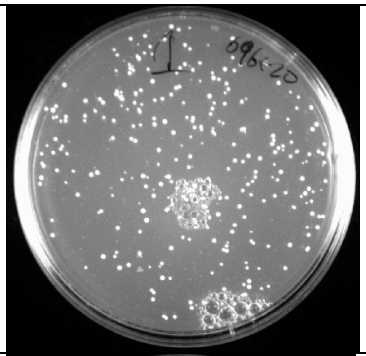
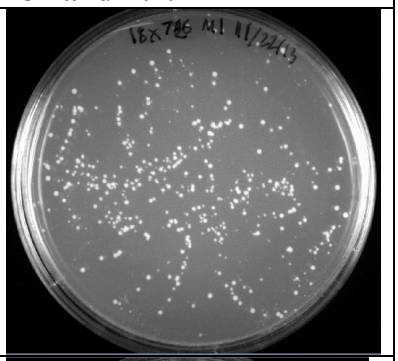
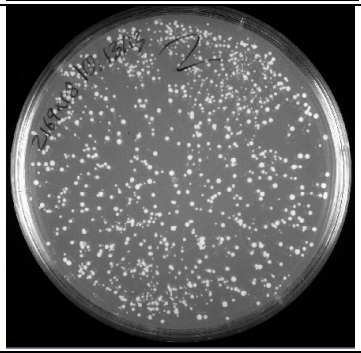
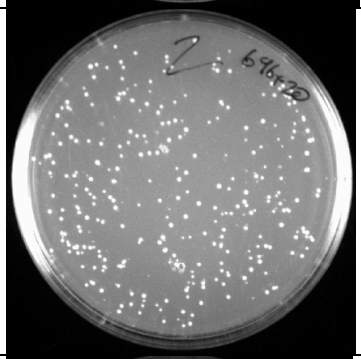
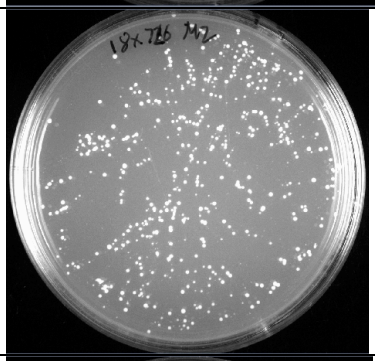
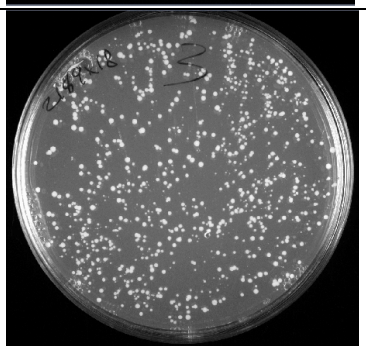
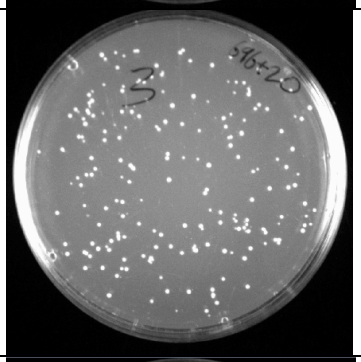
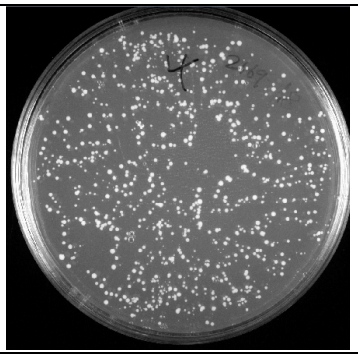
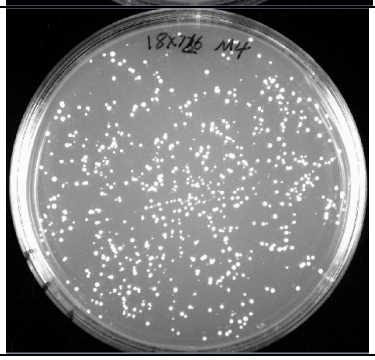
	2169 and *Bim1Δ*	**423 and *Bik1Δ***	**432 and *Bim1Δ***
Plate 1: Non-selective			
Plate 2: + G418 Selects for spores w/ *Bik1Δ/Bim1Δ*)::KanMX			
Plate 3: -Uracil Selects for spores w/ *Tub2*::URA			
Plate 4:+G418,-Uracil selects for spores w/ *Bik1Δ/Bim1Δ*)::KanMX and *Tub2*::URA			
	No Interaction	SS	SE

Figure 9: This figure shows typical RSA plates for *tub2* mutants that have either no interaction, a synthetic sensitive (SS) interaction or synthetic enhanced (SE) interaction. The 2169 strain has a wildtype *TUB2* gene and served as a control.

Table 10: Association of Synthetic Interactions with Cold Sensitivity, Benomyl Sensitivity/Resistance and Colony Sickness

Bim1	**CS (dec.)**	**Ben-R (dec.)**	**Ben-SS (dec.)**	**Sick (dec.)**
No Interaction (6)	1 (0.17)	2 (0.33)	4 (0.67)	0 (0)
SS (11)	8 (0.73)	3 (0.27)	5 (0.45)	2 (0.18)
SS<.15 (8)	6 (0.75)	2 (0.25)	4 (0.5)	1 (0.13)
SS>.15 (3)	2 (0.67)	1 (0.33)	1 (0.33)	1 (0.33)
SE (3)	0 (0.00)	2 (0.67)	0 (0)	0 (0)
Total (20)	9 (0.45)	7 (0.35)	9 (0.45)	2 (0.1)
P-value	p=0.184	p=0.86	p=0.71	p=0.50

Bik1	**CS (dec.)**	**Ben-R (dec.)**	**Ben-SS (dec.)**	**Sick (dec.)**
No Interaction (10)	1 (0.10)	3 (0.3)	6 (0.6)	0 (0)
SS/SL (11)	9 (0.82)	4 (0.36)	4 (0.36)	2 (0.18)
SS/SL<.15 (8)	7 (0.88)	4 (0.5)	2 (0.25)	1 (0.13)
SS>.15 (3)	2 (0.67)	0 (0)	2 (0.67)	1 (0.33)
SE (1)	0 (0.00)	1 (1)	0 (0)	0 (0)
Total (22)	10 (0.45)	8 (0.36)	10 (0.45)	2 (0.09)
P-value	**p=0.043**	p=0.60	p=0.70	p=0.40

Table 10: Row labels: SS=Synthetic Sensitive, SS<0.15 = Synthetic Sensitive where the colony ratio of plate 4 is <0.15 SS<0.15 = Synthetic Sensitive where the colony ratio of plate 4 is >0.15 and SE=Synthetic Enhanced. Column labels: CS=Cold Sensitivity, Ben-R=Benomyl Resistance, Ben-SS= Benomyl Super Sensitive and Sick=Colony Sickness.
P-values were calculated using a X^2 test ($p<0.05$), the p=0.043 for *BIK1* suggests a non-random association between *BIK1* and *TUB2* synthetic interactions and cold sensitivity. A higher than expected proportion of SS/SL strains were cold sensitive.

Figure 11: Visualizations of β-tubulin showing location of *tub2 mutations*

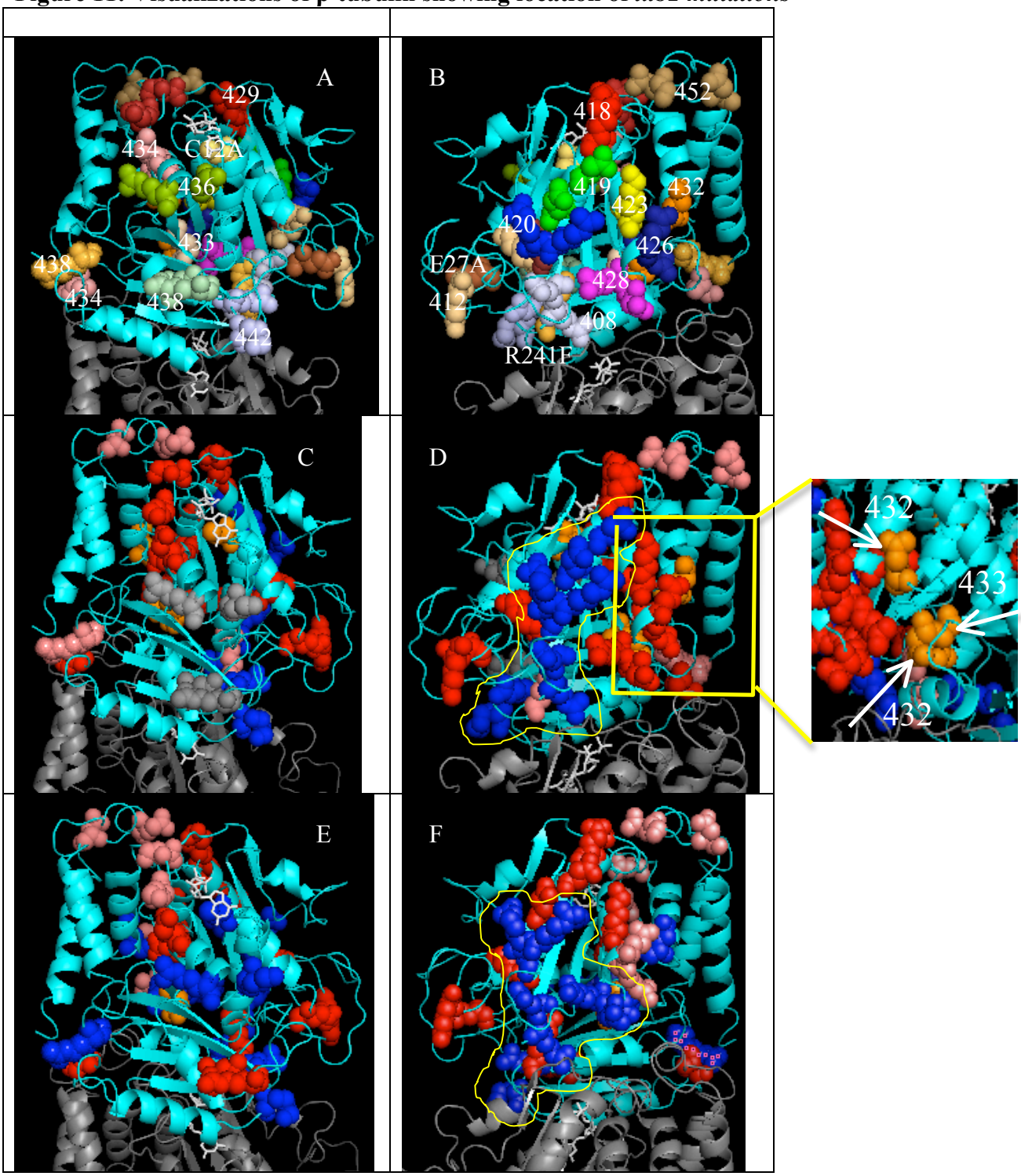


Figure 11: Panels A and B show 180° perspectives of a pymol model of β-tubulin (teal) with α-tubulin shown in grey. The mutated amino acids are represented as spheres with each color representing a different *tub2* mutation. Panels C and D show the mutations grouped by the type of synthetic interaction that occurred with *BIM1*. The thin yellow line shows where no interaction alleles have clustered together. Panel D also includes a blowup of two adjacent SE mutations. Blue= No interaction, Red= Synthetic Sensitive w/ CR of P4<0.15, Salmon=Synthetic Sensitive w/CR of P4>0.15, Orange=Synthetic Enhanced Panels E and F show the same as panels C and D except the interactions were with *BIK1*.